Phytochemistry, Vol 27, No 11, pp 3649–3652, 1988
Printed in Great Britain

0031-9422/88 $3.00+0.00
Pergamon Press plc

ALKALOIDS OF *AMSONIA BREVIFOLIA*

PERVEEN SHARMA*, YOSHIAKI SHIRATAKI† and GEOFFREY A. CORDELL ‡

Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612, U.S.A

(*Received* 7 *April* 1988)

Key Word Index—*Amsonia brevifolia*, Apocynaceae; oxindole alkaloids; indole alkaloids; cytotoxicity

Abstract—Two new cytotoxic oxindole alkaloids, 17-demethoxy corynoxine B and 17-demethoxy-isorhynchophylline were isolated from the whole plant of *Amsonia brevifolia* and their structures elucidated through a combination of physical, chemical and spectroscopic methods. Several terpenoids and nine other indole alkaloids were also isolated Because the constituents of *A. brevifolia* and *A. tomentosa* appeared to be identical qualitatively and quantitatively, it is suggested that only the name *A. brevifolia* be retained for this species.

INTRODUCTION

As part of our continuing program on the study of plant anticancer agents, extracts of the whole plant material of *Amsonia brevifolia* A. Gray and *Amsonia tomentosa* Torrey et Gray (Apocynaceae) [1] were found to display cytotoxic activity in the P-388 test system *in vitro* [2, 3]. Our initial aim was therefore to determine through bioactivity directed fractionation, the nature of the cytotoxic principles.

Amsonia brevifolia and *A tomentosa* are native to the southwestern part of the U.S.A and are recognized to be morphologically very close, differing only in the presence of surface hairyness. There have been no phytochemical reports on these two species and therefore a secondary aim of the present study was a chemotaxonomic comparison of the alkaloidal constituents. In this paper therefore we also report on the isolation and structure elucidation of the alkaloids of *A. brevifolia* and a comparison of the alkaloid profile of *A. brevifolia* and *A. tomentosa*.

RESULTS AND DISCUSSION

Fractionation of the alkaloid extract from the whole plant part afforded, in addition to a number of known, biogenetically related indole alkaloids, two alkaloids which from their UV chromophore (λ_{max} 214, 253 and 286 nm) contained an unsubstituted oxindole moiety. The occurrence of oxindole alkaloids in the Apocynaceae is extremely rare [4, 5] and we were therefore interested in their structure and relationship to the other alkaloids.

Alkaloid A was obtained as fine colourless needles, mp 178–180°, $[\alpha]_D$ +61°, $[M]^+$ 354 analysing for $C_{21}H_{26}N_2O_3$, and, from its IR spectrum gave evidence for lactam (3201 and 1678 cm^{-1}) and α,β-unsaturated ester moieties (1718 and 1622 cm^{-1}). The ^1H NMR spectrum displayed four aromatic protons in the region δ 6.92–7.29, a three-proton triplet at δ 0.21 for the C-18 protons, a methoxycarbonyl singlet, two olefinic protons at δ 6.23 and 5.62, an ethyl group and an α-acrylic ester unit. The D_2O-exchangeable lactam proton was observed at 8.25 ppm. Biogenetically these data are best explained in terms of the skeletal framework shown in **1**

Alkaloid B was obtained as an amorphous gum $[\alpha]_D$ 0°, M^+ 354 and was isomeric with alkaloid A. The IR, UV, ^1H NMR and ^{13}C NMR spectral data were extremely similar to those of alkaloid A suggesting that alkaloid B had the same gross structure as alkaloid A, but that it differed at one or more stereochemical centers

If one assumes that the stereochemistry at C-15 in these alkaloids is the same as that in all other non-rearranged monoterpene indole alkaloids [6], there are three remaining asymmetric centres to be assigned (C-3, C-7 and C-20). The problems of determining such stereochemical assignments are well known [7, 8] and are not trivial. In this instance, the chemical [9] and spectroscopic [8] procedures used for the stereochemical analysis of the rhynchophylline type alkaloids were employed.

Acid isomerization of either alkaloid A or alkaloid B, followed by TLC analysis indicated the presence of the original compound and an isomerization product. No interconversion of the two alkaloids was observed, thereby eliminating the possibility that the alkaloids had the same stereochemistry at C-20. Rather it suggested that one possesssed the *allo* and the other the *normal* configuration [9].

In the ^1H NMR spectrum of alkaloid B, a partly resolved C-18 Me triplet was observed at 0.49 indicating a *normal* configuration at C-20. On the other hand, the same resonance in alkaloid A was displayed as a symmetrical triplet at δ 0.21. The shielding of this group is due to close proximity of the N_4-lone pair with an axial C-20 ethyl group [9]. Also observed in the ^1H NMR spectrum of alkaloid B was a downfield doublet at δ 7.51 for H-9, indicating a *normal* A configuration [8]. By

*Present address. Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Texas, Houston, TX 77030, U.S.A.

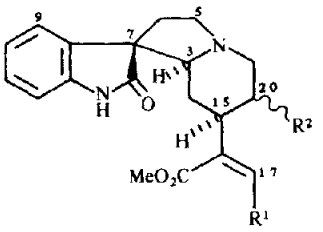
†Present address: Josai University, Keyakidai, Sakado, Saitama 350-02, Japan.

‡Author to whom correspondence should be addressed.

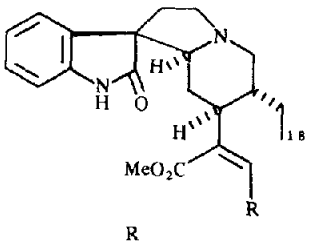
contrast, H-9 in the spectrum of alkaloid B is not very distinguishable from the other aromatic protons, appearing at δ 7.19. An *allo* B configuration is therefore indicated for alkaloid A [8].

The chemical shift values of C-3 and C-9 are also strongly diagnostic of the configuration of the spiro carbon (C-7) in the rhynchophylline type alkaloids [8]. Thus for the A series of alkaloids the values are δ 72.2 and 125.2, respectively and for the B series the corresponding values are δ 75.3 and 122.9 ppm respectively. Comparison of the data for alkaloid A (δ 74.4 and 122.6) and alkaloid B (δ 71.9 and 124.8) with values for isorhynchophylline (**2**) and rhynchophylline (**3**) indicates that alkaloid A has the B configuration and alkaloid B has the A configuration.

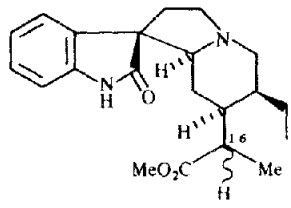
The CD spectra of alkaloids in this series are also important [9–12]. In alkaloid B a negative Cotton effect was observed at 288 nm and a positive Cotton effect at 210 nm, substantiating an A configuration at C-7. By contrast, alkaloid A displayed a positive Cotton effect at 290 nm and a negative Cotton effect at 210 nm (B configuration at C-7). Similar Cotton effects have been noted previously for isorhynchophylline (**2**) and corynoxine B (**3**) [9, 12]. The C-3Hα configuration for alkaloids A and B was deduced from the observation of negative Cotton effects at 262 nm for both alkaloids [10, 11], and the overall similarities to isorhynchophylline (**2**) and corynoxine B (**4**) confirmed the stereochemistry at C-15. Thus alkaloid B has the absolute configuration 3*S*,4*R*,7*S*,15*S*,20*R* and the structure 17-demethoxy-isorhynchophylline (**5**). Alkaloid A on the other hand has the absolute configuration 3*S*,4*R*,7*R*,15*S*,20*S* and the structure 17-demethoxy corynoxine B (**1**). Either catalytic or sodium borhydride reduction of **1** led to a mixture of 16,17-dihydro products, **6** and **7**, which were characterized, but not stereochemically defined.



	R^1	R^2
1	H	β-C_2H_5
3	OMe	-C_2H_5
4	OMe	-C_2H_5



	R
2	OMe
5	H



6/7

These two alkaloids are therefore closely related to catharinensine, an oxindole alkaloid isolated from *Peschiera catharinensis* (DC.) Miers (Apocynaceae), which was assigned the stereochemistry 3*R*,4*S*,7*R*,15*R*,20*S* because its CD spectrum was the mirror image of corynoxine (**3**) (*allo* A) [5]. 17-Demethoxy-isorhynchophylline (**5**) was obtained as a synthetic product from rhynchophylline (**3**) during these studies [15]. Given the well established biosynthesis of indole alkaloids and the resulting stereochemistry at C-15 which remains as the *S* configuration in the alkaloids which have not passed through a secodine intermediate, the relationship of catharinensine to our isolate 17-demethoxy corynoxine B remains unclear.

Also isolated in this study were the terpenoids ursolic acid and the ubiquitous β-sitosterol-β-D-glucoside, and the alkaloids tetrahydroalstonine, vallesiachotamine, isovallesiachotamine, picralinal, picrinine, akuammidine, tabersonine, tubotaiwine and akuammicine. Their structures were determined through comparison with literature data or by direct comparison with authentic samples.

Ursolic acid and four of the alkaloids, tabersonine, vallesiachotamine, and the new alkaloids **1** and **5** were active in the P-388 lymphocytic leukemia test system *in vitro* according to established protocols [2, 3].

In order to determine the taxonomic relationship between *A. brevifolia* and *A. tomentosa*, the pet ether, methanol, neutral/acidic and basic alkaloid fractions were examined by TLC on three different solvent systems using four different spray reagents. Each of the corresponding fractions from each plant was identical, indicating that the species are chemotaxonomically indistinguishable. It is suggested therefore that only the name *Amsonia brevifolia* A. Gray should be retained for this species.

EXPERIMENTAL

Mp: uncorr IR spectra were determined as KBr discs on a Nicolet MX-1 interferometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Nicolet NMC 360 spectrometer using TMS as an internal standard. Chemical shifts are recorded in δ ppm units. Mass spectra were obtained with a Varian MAT 112S double focussing spectrometer operating at 70 eV. Analtech silica gel plates were used for both analytical and prep. TLC. Solvent systems used for preparative TLC were A (EtOAc–C_6H_6, 1:1), B ($CHCl_3$–MeOH, 9:1) and C ($CHCl_3$–MeOH, 5:1).

Plant material. A. brevifolia A. Gray and *A. tomentosa* Torrey et Gray were collected in the Mojave Desert, California in April 1983. The plant samples were identified by Dr J. Henrickson (California State University, Los Angeles, CA) and samples are deposited in the herbarium of the Field Museum of Natural History, Chicago, IL, U.S.A.

Fractionation of A. brevifolia. The powdered, air-dried whole plant of *A. brevifolia* (6 kg) was exhaustively extd with petrol (3

× 20 l) at room temp. and the total extract was concd *in vacuo* to afford a residue (401 g) The marc was extd with MeOH (4 × 20 l) and the combined exts were concd *in vacuo* to yield a residue (1200 g). A portion of the MeOH ext (600 g) was treated with 2% tartaric acid (3 l), filtd and extd with $CHCl_3$ (5 × 200 ml) The $CHCl_3$ ext was washed with H_2O, dried (Na_2SO_4) and concd to a neutral/acidic fraction A (54 g) Na_2CO_3 soln (10%) was added to the aq. layer to pH 9 and extd with EtOAc. The EtOAc ext was washed with H_2O, dried (Na_2SO_4) and concd *in vacuo* to afford fraction B (11 5 g)

Fractionation of A tomentosa. The air-dried whole plant of *A tomentosa* (500 g) was successively extd with petrol and MeOH at room temp After evpn of the exts *in vacuo*, the residues weighed 30 and 120 g, respectively. The MeOH ext was treated with 2% tartaric acid and worked-up as described above to afford a neutral/acidic fraction A (1 02 g) and a basic fraction B (1 0 g).

TLC comparison of A brevifolia *and* A. tomentosa A comparative TLC study of the petrol, MeOH ext. fraction A and fraction B from *A brevifolia* and *A tomentosa* was carried out using H_2SO_4 Dragendorff, Ce^{3+} and $FeCl_3$ chromogenic reagents. These studies showed that the chemical consts of *A brevifolia* and *A tomentosa* were the same in the three different solvent systems A, B and C Fractions A and B from *A. tomentosa* were subjected to column and prep chrom over silica gel. TLC studies of isolated constituents revealed complete similarity to those isolated from *A brevifolia*

Isolation of constituents from Amsonia brevifolia. The basic alkaloid fraction (11 5 g) from *A. brevifolia* was chromatographed on a column of silica gel (600 g) and eluted with $CHCl_3$ and $CHCl_3$–MeOH mixtures of increasing polarity A total of 35 fractions were collected and the following isolates obtained as indicated fraction 3, prep TLC solvent A, tetrahydroalstonine, 30 mg, 0 001% [13–15], fractions 4–9, prep TLC, solvent A, isovallesiachotamine, 10 mg, 0 0003% [16]; fractions 4–9, prep TLC, solvent A, vallesiachotamine, 20 mg, 0 006% [16]; fraction 17, cryst picralinal, 50 mg, 0 017% [17–19], fractions 19–20, cryst picrinine, 135 mg, 0.046% [20, 21]; fractions 21–22, cryst, akuammidine, 200 mg, 0 007% [22]

Fraction A (54 g) was separated on silica gel (800 g) eluting with increasingly polar mixtures of $CHCl_3$–MeOH to afford ursolic acid, fraction 3, chrom silica gel, tabersonine, 65 mg, 0 0023% [23, 24], fraction 12, prep TLC, solvent C, tubotaiwine, 6 mg, 0 0002% [25], fraction 12, prep TLC, solvent C, akuammicine, 15 mg, 0.0005% [21, 26]

Isolation of 17-*demethoxy-isorhynchophylline* (**5**) *and* 17-*demethoxy-corynoxine B* (**1**). Fractions 10–13 from the chromatography of fraction A eluted with $CHCl_3$–MeOH (49 1) yielded a residue which was subjected to prep TLC eluting with solvent B to afford a gummy solid of alkaloid B, **5**, (60 mg, 0 002%) having the following physical and spectroscopic properties. $[\alpha]_D$ 0° (MeOH; *c* 0 2), UV λ_{max}^{MeOH} nm, 210, 252, 282, IR ν_{max}^{KBr} cm^{-1}; 3210, 2963, 2884, 1719, 1707, 1622, 1471, 1208, 1192, 1159, 1142, 752, 732, CD (MeOH) (2 7 mg/25 ml) $[\theta]_{238}+19{,}300$, $[\theta]_{262}-5{,}570$, $[\theta]_{290}+6230$; ^{1}H NMR ($CDCl_3$) δ 0 49 (*t*, 3H, *J* = 7 2 Hz, 18-H_3), 0.57 (*m*, 1H, H-19), 0 89 (*q d*, 1H, *J* = 7 2, 7 2, 14 4 Hz, H-19), 1 65 (*m*, 2H, H-20 and H-21α), 1 75 (*dd*, 1H, *J* = 9 6, 3 0 Hz, H-14α), 2.03 (*m*, 1H, H-6α), 2 18 (*br t* 1H, H-14β), 2 35 (*ddd*, 1H, *J* = 7, 3 Hz, H-6β), 2 54 (*m*, 2H, H-15 and H-5α), 2 62 (*br d*, 1H, *J* = 10 2 Hz, H-21β), 3 19 (*br d*, 1H, *J* = 10 6 Hz, H-3), 3.27 (*br t*, 1H *J* = 8.3 Hz, H-5β), 3.69 (*s*, 3H, OMe), 5 45 (*s*, 1H, H-17α), 6.16 (*s*, 1H, H-17β), 6 90 (*d*, 1H, *J* = 7 6 Hz, H-12), 7 05 (*d t*, 1H, *J* = 7 5, 0.6 Hz, H-10), 7 19 (*d t*, 1H, *J* = 7 5, 0 6 Hz, H-11), 7.51 (*d*, 1H, *J* = 7 6 Hz, H-9) and 9.42 (*br s*, 1H, D_2O exchanged, NH), ^{13}C NMR ($CDCl_3$) δ 182 4 (C-2), 167.5 (C-22), 142.6 (C-16), 140.2 (C-13), 135 4 (C-8), 127 5 (C-11), 125 2 (C-17), 122 6 (C-9 and C-10), 109 9 (C-12), 74 4 (C-3), 55 4 (C-7), 54 7 (C-21), 52 8 (C-5), 51 7 (OMe), 40.8 (C-15 or C-20), 39 8 (C-20 or C-15), 37 7 (C-6), 33 4 (C-14), 19 5 (C-19) and 7 9 (C-18); MS, *m/z* (rel. int.) 354 (M^+, 79), 339 (4), 325 (4), 323 (6), 295 (2), 283 (4), 209 (62), 208 (63), 194 (100), 180 (53), 159 (10), 130 (16), 129 (17), 82 (20), 69 (16) and 41 (34).

Fraction 15 eluted with $CHCl_3$-MeOH (98 2) was evapd and the residue cryst from EtOAc as colourless fine needles of alkaloid A, **1** (80 mg, 0 0027%), mp 178–80°, $[\alpha]_D$ +61° (MeOH, *c* 0 2), UV λ_{max}^{MeOH} 214, 253, 286 nm, IR ν_{max}^{KBr} 3201, 3190, 3184, 1713, 1678, 1622, 1478, 1271, 750 cm^{-1}; CD (MeOH) (0.6 mg/25 ml) $[\theta]_{238}+23900$, $[\theta]_{262}-9{,}730$, $[\theta]_{288}-7{,}670$, ^{1}H NMR ($CDCl_3$) δ 0 21 (*t*, 3H, *J* = 7 5 Hz, 18-H_3), 0 66 (*q d*, 1H, *J* = 7.6, 7 6, 14 4 Hz, C-19), 1 07 (*q d*, 1H, *J* = 3 6 Hz, H-19), 1 71 (*m*, 2H, H-14 and H-21), 1 95 (*m*, 1H, H-6), 2.11 (*m*, 2H, H-14 and H-20), 2.51 (*m*, 4H, H-6, H-21, H-5 and H-15), 3 27 (*t d*, 1H, *J* = 3 6, 3.6, 10.4 Hz, H-3), 3 34 (*m*, 2H, H-14 and H-21), 3.71 (*s*, 3H, OMe), 5 61 (*s*, 1H, H-17), 6 23 (*s* 1H, H-17), 6 93 (*br d*, 1H, *J* = 7.7 Hz, H-12), 7 05 (*d* t, 1H, *J* = 7 6 0.7 Hz, H-10), 7.19 (*d t*, 1H, *J* = 7 6, 0 7 Hz, H-11), 7 28 (*br d*, 1H, *J* = 7 7 Hz, H-9) and 8 82 (*br s*, 1H, D_2O exchanged, NH); ^{13}C NMR ($CDCl_3$) δ 183 4 (C-2), 167 3 (C-22), 142 6 (C-16), 140 3 (C-13), 133 9 (C-8), 127 4 (C-11), 125 2 (C-17), 124 8 (C-9), 122 3 (C-10), 109 7 (C-12), 71 9 (C-3), 56.2 (C-7), 53.8 (C-21), 52 7 (C-5), 51.7 (OMe), 40 6 (C-15 or C-20), 40.1 (C-20 or C-15), 37.3 (C-6), 33 4 (C-14), 19.5 (C-19) and 7 7 (C-18); MS, *m/z* (rel. int) 354 (M^+, 56), 339 (3), 325 (4), 323 (4), 282 (8). 209 (49), 208 (45), 194 (100), 180 (38), 83 (44) and 69 (10); mass measurement found 354 1922, calcd for $C_{21}H_{26}N_2O_3$ 354 1943.

Catalytic reduction of **1**. Alkaloid **1** (20 mg) in MeOH (40 ml) was hydrogenated at 35–40 psi over Pd-C (20 mg) for 5 hr The reaction mixture was filtered, concd and the residue subjected to prep TLC in solvent B to afford two products, reduction product A (**6**) was obtained as a colourless amorphous solid. UV λ_{max}^{MeOH} nm 208, 253, 282, NMR ($CDCl_3$) δ 0.35 (*t*, 3H, *J* = 7.5 Hz, 18-H_3), 0 84 (*q d*, 1H, *J* = 3 8 Hz, H-19), 1 01 (*d*, 3H, *J* = 4, 2 8 Hz, 17-H_3), 1 16 (*q d*, 1H, *J* = 4 0 Hz, H-19), 2 45 (*dd*, *J* = 4, 2 8 Hz), 2 64 (*q d*, 1H, *J* = 3.6 Hz, H-16), 3 65 (*s*, 3H, OMe), 6.88 (*d*, 1H, *J* = 7 7 Hz, H-12), 7 05 (*d t*, 1H, *J* = 0 8, 7 8 Hz, H-10), 7 19 (*d t*, 1H, *J* = 0 8, 7 8 Hz, H-11), 7 27 (*d*, *J* = 7 5 Hz, H-9) and 8 04 (*br s*, 1H, NH); MS, *m/z* (rel. int.) 356 (M^+, 27), 325 (4), 297 (1), 269 (17), 239 (2), 211 (4), 210 (21), 196 (11), 183 (11), 182 (100), 95 (15) and 41 (41)

Reduction product B (**7**) was obtained as a colourless glass (2.2 mg), UV λ_{max}^{MeOH} 208, 252, 283 nm, NMR ($CDCl_3$) δ 0.26 (*t*, 3H, *J* = 7.2 Hz, 18-H_3), 0 57 (*q d*, 1H, *J* = 3 5 Hz, H-19), 1 12 (*d*, 3H, *J* = 7 5 Hz, 17-H_3), 1 40 (*q d*, 1H, *J* = 3 5 Hz, H-19), 2.71 (*q d*, 1H, *J* = 3 6 Hz, H-16), 3 59 (*s*, 3H, OMe), 6 88 (*d*, 1H, *J* = 7.8 Hz, H-12), 7.04 (*t d*, 1H, *J* = 0 9, 7 3 Hz, H-10), 7 18 (*t d*, 1H, 0 9, 7.3 Hz, H-11), 7 26 (*d*, 1H, *J* = 7 2 Hz, H-9) and 8 21 (*br s*, 1H, D_2O exchanged, NH), MS, *m/z* (rel int) 356 (M^+, 37), 325 (61), 297 (5), 269 (19), 239 (3), 211 (21), 210 (28), 196 (16), 183 (15), 182 (100), 130 (16), 124 (28), 123 (16) 95 (19), 55 (30), 42 (32) and 41 (41)

$NaBH_4$ reduction of **1**. Alkaloid **1** (15 mg) was dissolved on MeOH (10 ml), cooled to −10° and stirred with $NaBH_4$ (25 mg) at −10° for 2 hr The mixture was worked-up in the usual way to afford two products **6** and **7** which were identical with the products obtained through catalytic hydrogenation

Biological testing Isolates were tested in the KB and P-388 test systems *in vitro* according to established protocols [2, 3] ED_{50} (μg/ml) (KB, P-388), tabersonine 2.3, 0.88, ursolic acid 4 4, 6.4; tubotaiwine 10 8, 50; akuammicine 50, 50, tetrahydroalstonine 50, 50; vallesiachotamine 1.9, 4 2 [27], 17-demethoxyisorhynchophylline (**5**) 2 3, 2 6, 17-demethoxy corynoxine B (**1**) 2.6, 2.8, picralinal 17 9, 50, picrinine 50, 50, akuammidine 50, 50

Acknowledgements—This work was supported, in part, by grant CA-20164 from the Division of Cancer Treatment, National

Cancer Institute, Bethesda, MD We also thank the Research Resources Center of the University of Illinois at Chicago for the provision of nuclear magnetic resonance and mass spectormetric facilities, Dr D D Soejarto for making arrangements for the collection and identification of the plant material, Dr J M Pezzuto for the biological testing, Professor J D Phillipson (University of London) for helpful discussions and the provision of relevant sections of ref 8, and Professor A J Marsaioli (Universidade Estadual de Campinas, Campinas Brasil) for spectroscopic data of catharinensine

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